



ISSN Print: 2618-060X
 ISSN Online: 2618-0618
 NAAS Rating (2026): 5.20
 IJRA 2026; 9(10): 611-617
www.agronomyjournals.com
 Received: 30-07-2026
 Accepted: 25-09-2026
 Published: 10-10-2026

The names and affiliations of all authors are provided below, following the references.

Comparative study on detection, isolation and identification of seed-borne mycoflora in different varieties of French bean

Kamble AB¹, Magar SJ², Hande VS³, Pathak DE⁴, Tandle VH⁵, Patil NH⁶, Ovhal SS⁷

DOI: <https://www.doi.org/10.33545/2618060X.2026.v9.i10h.6857>

ABSTRACT

The present investigation was undertaken to detect, isolate and identify seed-borne mycoflora associated with French bean (*Phaseolus vulgaris* L.) seeds using different seed health testing techniques. Four French bean varieties, viz., Rajma Red Small, Rajma Kashmiri, Phule Varun and Phule Rajma, were evaluated using the standard blotter paper method, standard agar plate method, modified potato dextrose agar (PDA) method and rolled towel paper method. A total of 400 seeds of each variety were tested for seed germination, healthy and infected seeds and associated seed-borne fungi. The standard blotter paper method recorded mean germination of 77.25 per cent and mean seed mycoflora of 8.31 per cent, whereas the standard agar plate method recorded 84.25 and 13.73 per cent, respectively. The modified PDA method recorded 87.00 per cent mean germination and the highest mean seed mycoflora of 15.98 per cent, while the rolled towel paper method recorded 87.00 per cent germination and 8.87 per cent mean seed mycoflora. The seed-borne fungi isolated and identified included *Colletotrichum lindemuthianum*, *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma harzianum*, *Penicillium* sp. and *Macrophomina phaseolina*. Among the detected fungi, *A. niger* and *F. oxysporum* were consistently associated with French bean seeds across the different testing methods. The modified PDA method detected a comparatively wider range of seed-borne fungi, including *C. lindemuthianum*, *A. flavus* and *T. harzianum*, which were not detected by some of the other methods. Morphological characterization of the isolated fungi was carried out based on colony characteristics, mycelial growth, conidial morphology and other diagnostic structures. The findings demonstrated considerable variation among seed health testing techniques in the detection and recovery of seed-borne mycoflora. The modified PDA and rolled towel paper methods were useful for assessing seed health and associated fungal infection, while the isolation and identification studies confirmed the occurrence of economically important seed-borne fungi in French bean. The study provides useful information for selecting appropriate seed health testing techniques and for further investigations on the pathogenicity and management of important seed-borne pathogens of French bean.

Keywords: French bean, *Phaseolus vulgaris*, Seed-borne mycoflora, Seed health testing, Isolation, Identification, Blotter paper method, PDA method, Rolled towel paper method

Introduction

French bean (*Phaseolus vulgaris* L.) is an important legume crop valued for its nutritional and economic significance. Good quality and healthy seed are essential for rapid germination, vigorous seedling growth and uniform crop establishment. However, seeds may harbour several pathogenic and saprophytic fungi that reduce seed quality, germination and seedling vigour and may serve as primary sources of infection.

Several fungi, including *Colletotrichum lindemuthianum*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Aspergillus* spp., *Penicillium* sp., *Cladosporium* sp. and *Alternaria* sp. have been reported in association with French bean seeds. Accurate detection and identification of these seed-borne fungi is therefore important for assessing seed health and developing suitable management strategies.

Different seed health testing techniques, such as the standard blotter paper, standard agar plate, modified potato dextrose agar (PDA) and rolled towel paper methods, vary in their efficiency for detecting and recovering seed-borne fungi. Hence, the present study was undertaken to detect, isolate and identify seed-borne mycoflora associated with French bean (*Phaseolus vulgaris* L.) and to compare the efficiency of different seed health testing techniques.

Corresponding Author:

Kamble AB

PG Scholar, Department of Plant Pathology, College of Agriculture, Latur VNMKV, Parbhani, Maharashtra, India

2. Materials and Methods

2.1 Experimental material

Four French bean varieties, namely Rajma Red Small, Rajma Kashmiri, Phule Varun and Phule Rajma, were used for detection of seed-borne mycoflora. Four hundred seeds of each variety were tested using different seed health testing techniques.

2.2 Seed health testing techniques

The seed-borne mycoflora were detected using four methods, viz., standard blotter paper method, standard agar plate method, modified PDA method and rolled towel paper method, following the International Seed Testing Association rules (Anonymous, 1999).

2.2.1 Standard blotter paper method

Seeds were incubated on moist blotter paper discs at $27 \pm 2^\circ\text{C}$ for seven days under alternate cycles of 12 h light and 12 h darkness. The fungal colonies developing on the seeds were examined under a stereo-binocular microscope. The fungal colonies were identified based on their morphological characteristics and recorded.

2.2.2 Standard agar plate method

Seeds were placed on sterilized potato dextrose agar (PDA) medium at the rate of ten seeds per plate and incubated at $27 \pm 2^\circ\text{C}$ for seven days. The fungal colonies developing from the seeds were examined isolated and identified based on their morphological characteristics.

2.2.3 Modified PDA method

For the modified PDA method, seeds were surface sterilized with 1 per cent sodium hypochlorite solution for two minutes and subsequently washed three times with sterile distilled water. The treated seeds were then plated on PDA medium and incubated under suitable conditions for fungal development. The associated fungi were examined and identified based on their morphological characteristics.

2.2.4 Rolled towel paper method

The rolled towel paper method was used to assess seed germination, seedling quality and associated seed mycoflora. Randomly selected 400 seeds were placed between moist germination papers, rolled and

incubated in a slanting position at $27 \pm 2^\circ\text{C}$. Observations on germination and seed mycoflora association were recorded after seven and ten days of incubation.

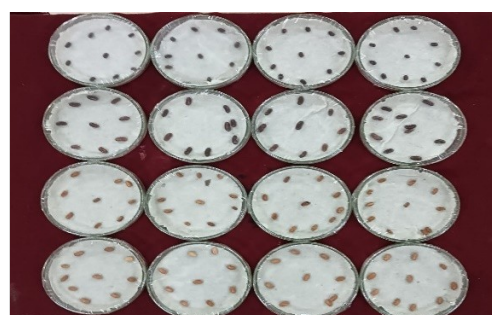
2.3 Isolation and identification of fungi

The fungal colonies developing from infected seeds were isolated and examined for their colony characteristics, mycelial growth, conidial morphology and other diagnostic structures. The associated fungi were identified as *Colletotrichum lindemuthianum*, *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma harzianum*, *Penicillium* sp. and *Macrophomina phaseolina*.

3. Results and Discussion

3.1 Detection of seed-borne mycoflora by standard blotter paper method

The standard blotter paper method recorded germination ranging from 65.00 per cent in Phule Varun to 84.00 per cent in Phule Rajma, with a mean germination of 77.25 per cent. The mean percentage of healthy and infected seeds was 79.12 and 20.37 per cent, respectively.



Seed detection by standard blotter paper method at 1 day

Seed detection by standard blotter paper method after 7 days

3.1 Detection of seed-borne mycoflora by standard blotter paper method

Table 3.1. Per cent frequency of seed-borne mycoflora of French bean seeds detected by standard blotter paper method

Sr. No.	Variety	Germination (%)	Healthy seeds (%)	Infected seeds (%)	Seed mycoflora (%)							
					<i>Cl</i>	<i>Fo</i>	<i>An</i>	<i>Af</i>	<i>Th</i>	<i>Ps</i>	<i>Mp</i>	Total %
1.	Rajma red Small	82.00	80.00	20.00	0.00	1.25	3.50	0.00	0.00	0.00	1.00	5.75
2.	Rajma Kashmiri	78.00	78.00	20.00	0.00	1.00	8.00	0.00	0.00	0.00	0.00	9.00
3.	Phule Varun	65.00	85.00	15.00	0.00	6.25	2.00	0.00	0.00	0.00	5.00	13.25
4.	Phule Rajma	84.00	73.50	26.50	0.00	1.25	4.00	0.00	0.00	0.00	0.00	5.25
Mean		77.25	79.12	20.37	0.00	2.43	4.37	0.00	0.00	0.00	1.50	8.31

Cl: *Colletotrichum lindemuthianum*; Fo: *Fusarium oxysporum*; An: *Aspergillus niger*; Af: *Aspergillus flavus*; Th: *Trichoderma harzianum*; Ps: *Penicillium* sp.; Mp: *Macrophomina phaseolina*

Table 4.1 presents the per cent frequency of seed-borne mycoflora associated with French bean seeds of four varieties as detected by the standard blotter paper method. The results revealed considerable variation in the incidence of seed-associated fungi among the varieties studied. The mean germination percentage was 77.25 per cent, while the mean healthy and infected seed percentages were 79.12 and 20.37 per cent, respectively. The total seed mycoflora ranged from 5.25 to 13.25 per cent, with an overall mean of 8.31 per cent.

Among the varieties, Phule Varun recorded the highest total seed mycoflora (13.25%), followed by Rajma Kashmiri (9.00%), Rajma Red Small (5.75%) and Phule Rajma (5.25%). The comparatively higher fungal incidence in Phule Varun indicates greater association of seed-borne fungi with this variety under the conditions of the present investigation. Such variation may be related to differences in seed characteristics, varietal response, seed maturity, storage conditions and the initial level of fungal contamination.



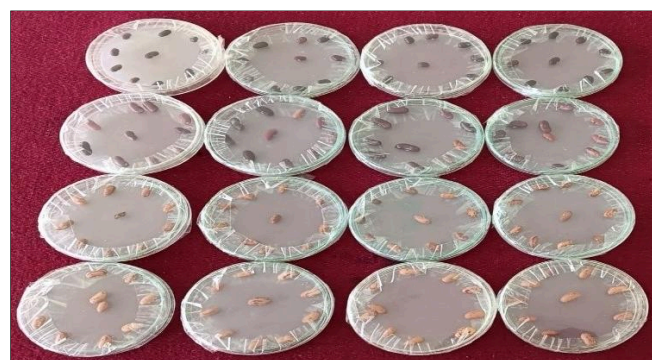
Among the fungi detected, *Aspergillus niger* was the predominant fungus, recording a mean frequency of 4.37 per cent, with the maximum incidence of 8.00 per cent in Rajma Kashmiri. *Fusarium oxysporum* was the next predominant fungus, with a mean frequency of 2.43 per cent, followed by *Macrophomina phaseolina* with 1.50 per cent. *Colletotrichum lindemuthianum*, *Aspergillus flavus*, *Trichoderma harzianum* and *Penicillium* sp. were not detected by the standard blotter paper method in the present samples.

The predominance of *A. niger* in the present study is in agreement with earlier investigations on French bean seed mycoflora. Sinha *et al.* (1999) [1] reported *A. niger* among the common fungi associated with French bean seeds and also found that the blotter method was effective for detecting seed-associated fungi. Similarly, Bhattacharjee *et al.* (2005) [2] reported *A. niger* and *F. oxysporum* among the fungi consistently associated with French bean seeds and observed that the blotter method was effective for isolation of seed mycoflora.

3.2 Detection of seed borne mycoflora by standard agar plate method

The standard agar plate method recorded mean germination of 84.25 per cent, while healthy and infected seeds averaged 74.75 and 25.25 per cent, respectively. Phule Varun recorded the highest infection of

30.50 per cent.



Seed detection by Standard agar plate method at 1 day



Seed detection by Standard agar plate method after 7 days

3.2 Detection of seed borne mycoflora by standard agar plate method

Table 3.2. Per cent frequency of seed-borne mycoflora of French bean seeds detected by standard agar plate method

Sr. No.	Variety	Germination (%)	Healthy seeds (%)	Infected seeds (%)	Seed mycoflora (%)							
					Cl	Fo	An	Af	Th	Ps	Mp	Total %
1.	Rajma red small	86.00	80.00	20.00	0.00	3.00	2.50	0.00	0.00	0.00	1.50	7.00
2.	Rajma Kashmiri	85.00	79.00	21.00	0.00	1.50	2.00	0.00	0.00	0.00	1.00	4.50
3.	Phule Varun	82.00	69.50	30.50	0.00	5.50	5.00	0.00	0.00	0.00	23.25	33.75
4.	Phule Rajma	84.00	70.50	29.50	0.00	8.20	0.00	0.00	0.00	0.00	1.50	9.70
Mean		84.25	74.75	25.25	0.00	4.55	2.37	0.00	0.00	0.00	6.81	13.73

Cl: *Colletotrichum lindemuthianum*; Fo: *Fusarium oxysporum*; An: *Aspergillus niger*; Af: *Aspergillus flavus*; Th: *Trichoderma harzianum*; Ps: *Penicillium* sp.; Mp: *Macrophomina Phaseolina*

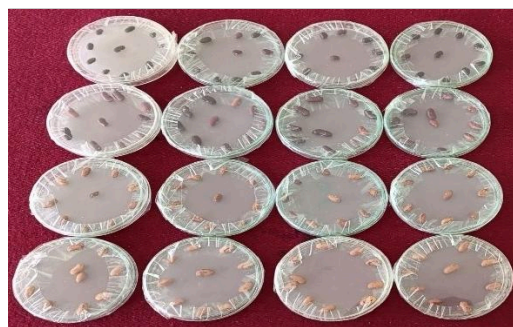
Aspergillus niger recorded a mean frequency of 2.37 per cent, while *F. oxysporum* recorded 4.55 per cent. *Macrophomina phaseolina* showed a mean frequency of 6.81 per cent and was particularly high in Phule Varun. The total seed mycoflora was highest in Phule Varun (33.75%), followed by Phule Rajma (9.70%), Rajma Red Small (7.00%) and Rajma Kashmiri (4.50%), with an overall mean of 13.73 per cent.

Sud *et al.* (2026) [3] reported considerable diversity of seed-borne fungi in kidney bean using agar plating and recovered fungi belong-

ing to several genera, including *Aspergillus*, *Colletotrichum*, *Fusarium*, *Penicillium* and *Trichoderma*.

3.3 Detection of seed borne mycoflora by modified PDA plate method

The modified PDA method recorded a mean germination of 87.00 per cent. Mean healthy and infected seeds were 45.00 and 55.00 per cent, respectively. Rajma Kashmiri and Phule Varun recorded comparatively higher infected seed percentages of 85.00 and 75.00 per cent, respectively.



Seed detection by modified PDA method at 1 day

Seed detection by modified PDA after 7 days

3.3 Detection of seed borne mycoflora by modified PDA plate method

Table 3.3. Per cent frequency of seed-borne mycoflora of French bean seeds detected by modified PDA method

Sr. No.	Variety	Germination (%)	Healthy seeds (%)	Infected seeds (%)	Seed mycoflora (%)							
					<i>Cl</i>	<i>Fo</i>	<i>An</i>	<i>Af</i>	<i>Th</i>	<i>Ps</i>	<i>Mp</i>	Total %
1.	Rajma red small	88.00	80.00	20.00	3.25	1.00	1.25	2.00	1.00	2.25	1.25	12.00
2.	Rajma Kashmiri	90.00	15.00	85.00	0.00	8.00	2.00	1.00	0.00	0.00	1.20	12.20
3.	Phule Varun	75.00	25.00	75.00	0.00	6.50	3.00	0.00	0.00	0.00	16.25	25.75
4.	Phule Rajma	95.00	60.00	40.00	5.00	5.50	1.00	0.00	0.00	1.25	1.25	14.00
Mean		87.00	45.00	55.00	2.06	5.25	1.81	0.75	0.25	0.87	4.98	15.98

Cl: *Colletotrichum lindemuthianum*; *Fo*: *Fusarium oxysporum*; *An*: *Aspergillus niger*; *Af*: *Aspergillus flavus*; *Th*: *Trichoderma harzianum*; *Ps*: *Penicillium sp.*; *Mp*: *Macrophomina phaseolina*

Among the seed-borne fungi, *F. oxysporum* recorded the highest mean frequency of 5.25 per cent, followed by *M. phaseolina* (4.98%), *C. lindemuthianum* (2.06%), *A. niger* (1.81%), *Penicillium sp.* (0.87%), *A. flavus* (0.75%) and *T. harzianum* (0.25%). The total seed mycoflora was highest in Phule Varun (25.75%), followed by Phule Rajma (14.00%), Rajma Kashmiri (12.20%) and Rajma Red Small (12.00%), with a mean of 15.98 per cent.

The modified PDA method detected a wider range of fungi than the standard blotter and agar plate methods. The occurrence of *C. lindemuthianum* and *F. oxysporum* is supported by the observations of Sud *et al.* (2026) [3], while similar observations regarding PDA-based recovery of seed-associated fungi have been reported by Kumar *et al.* (2023), Sharma and Singh (2022) and Patel *et al.* (2022).

**3.4 Detection of seed borne mycoflora by rolled towel paper method**

The rolled towel paper method recorded mean germination of 87.00 per cent and mean healthy and infected seed percentages of 45.00 and 55.00 per cent, respectively. *F. oxysporum* was the predominant fun-

gus, recording a mean frequency of 3.75 per cent, followed by *A. niger* (2.12%), *M. phaseolina* (1.75%), *A. flavus* (0.75%), *T. harzianum* (0.25%) and *Penicillium sp.* (0.25%). *C. lindemuthianum* was not detected by this method.

Roller towel paper method at 1 Day



Rolled towel paper method After 7 Days

3.4 Detection of seed borne mycoflora by rolled towel paper method

Table 3.4. Per cent frequency of seed-borne mycoflora of French bean seeds detected by rolled towel paper method

Sr. No.	Variety	Germination (%)	Healthy seeds (%)	Infected seeds (%)	Seed mycoflora (%)							
					<i>Cl</i>	<i>Fo</i>	<i>An</i>	<i>Af</i>	<i>Th</i>	<i>Ps</i>	<i>Mp</i>	Total %
1.	Rajma red small	88.00	80.00	20.00	0.00	1.00	2.50	2.00	1.00	1.00	2.00	9.50
2.	Rajma Kashmiri	90.00	15.00	85.00	0.00	8.00	2.00	1.00	0.00	0.00	1.00	12.00
3.	Phule Varun	75.00	25.00	75.00	0.00	2.00	3.00	0.00	0.00	0.00	3.00	8.00
4.	Phule Rajma	95.00	60.00	40.00	0.00	4.00	1.00	0.00	0.00	0.00	1.00	6.00
Mean		87.00	45.00	55.00	0.00	3.75	2.12	0.75	0.25	0.25	1.75	8.87

Cl: *Colletotrichum lindemuthianum*; *Fo*: *Fusarium oxysporum*; *An*: *Aspergillus niger*; *Af*: *Aspergillus flavus*; *Th*: *Trichoderma harzianum*; *Ps*: *Penicillium sp.*; *Mp*: *Macrophomina phaseolina*.

The total seed mycoflora was highest in Rajma Kashmiri (12.00%), followed by Rajma Red Small (9.50%), Phule Varun (8.00%) and Phule Rajma (6.00%), with a mean of 8.87 per cent.

Hussain *et al.* (2020) [4] reported considerable fungal contamination in French bean seed samples using standard blotter and agar plate methods and observed an association between higher fungal infection and reduced seed germination.

3.5 Comparative detection of seed-borne mycoflora

The four seed health testing techniques differed in their ability to detect and recover associated fungi. The modified PDA method recorded the highest mean seed mycoflora (15.98%), followed by the standard agar plate method (13.73%), rolled towel paper method (8.87%) and standard blotter paper method (8.31%). The modified PDA method detected all seven fungi recorded in the study and was particularly useful for detecting *C. lindemuthianum*, *A. flavus* and *T. harzianum*.

Aspergillus niger and *F. oxysporum* were consistently associated with French bean seeds across the different testing methods. The variation among methods indicates that the efficiency of detection depends on the growth and sporulation characteristics of individual fungi.

3.6 Isolation and identification of seed-borne fungi

The isolated fungi were identified based on their colony and microscopic characteristics.

Colletotrichum lindemuthianum produced greyish-white to pale cream colonies that later developed dark brown to blackish mucilaginous conidial masses. Conidia were hyaline, unicellular and oblong to cylindrical with rounded ends, while dark setae were associated with the acervuli.

Fusarium oxysporum produced white, cottony colonies with pinkish pigmentation on the reverse of the culture. Macroconidia were sickle-shaped and possessed three to four septa, while Chlamydospores were globose and sub globose arranged as short chains.

Aspergillus niger produced dark black colonies with black conidial heads. The conidia were globose, rough and dark coloured, with septate hyphae.

Aspergillus flavus produced yellowish-green powdery colonies. Conidiophores were erect and swollen at the apex, bearing numerous spherical to subspherical conidia.

Trichoderma harzianum showed rapid growth, initially appearing white and subsequently developing yellowish-green to deep-green colonies. The conidiophores were highly branched, with flask-shaped phialides bearing unicellular, pale-green conidia.



Pure culture of: *C. lindemuthianum*, *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma harzianum*, *Penicillium sp.*, *M. phaseolina*

Penicillium sp. initially produced white colonies that later became bluish-green. Septate hyphae and branched conidiophores bearing flask-shaped phialides and chains of conidia were observed.

Macrophomina phaseolina produced rapidly growing greyish-white colonies that became dark charcoal-grey to black with age. Numerous dark microsclerotia were observed throughout the culture.

4. Conclusion

The present investigation confirmed the occurrence of diverse seed-borne mycoflora associated with French bean seeds. Seven fungi, namely *Colletotrichum lindemuthianum*, *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma harzianum*, *Penicillium sp.* and *Macrophomina phaseolina*, were isolated and identified. The efficiency of detection varied considerably among the four seed health testing techniques. The modified PDA method recorded the highest mean seed mycoflora and recovered the widest range of fungi, whereas the standard blotter paper method recorded comparatively lower recovery. *A. niger* and *F. oxysporum* were consistently detected across the testing methods. The findings demonstrate the importance of employing suitable seed health testing techniques for reliable detection and isolation of seed-borne fungi in French bean and provide a basis for further pathogenicity and disease management studies.

References

1. Sinha A, Singh SK, Qaisar J. Seed mycoflora of French bean and its control by means of fungicides. *Der Tropenlandwirt*. 1999;100(1):59–67.
2. Bhattacharjee A, Dhar MS. Seed mycoflora and storage effect in the germination of French bean (*Phaseolus vulgaris* Linn.). *Journal of Mycology and Plant Pathology*. 2005;35:397–399.
3. Sud D, Sharma OP, Sharma PN. Seed mycoflora in kidney bean (*Phaseolus vulgaris* L.) in Himachal Pradesh. *Seed Research*. 2026;33(1):103–107.
4. Hussain S, Ajaib M, Asghar R, Ali I, Siddiqui MF. Mycoflora associated with *Phaseolus vulgaris* L. seeds and its impact on seed germination in Azad Jammu & Kashmir. *Pakistan Journal of Botany*. 2020;52(4):1455–1463.
5. Bagade AR, Giri GK, Shingne AW, Usendi PN. In vitro evaluation of fungicides, botanicals and bio-agents against *Colletotrichum lindemuthianum* causing anthracnose of bean. *International Journal of Current Microbiology and Applied Sciences*. 2020;9(8):3103–3110.
6. Bevilacqua V, Vitti A, Logozzo G, Marzario S, Gioia T, Nuzzaci M. Influence of cultivation areas on the seed-borne pathogens on two local common bean ecotypes of “Fagioli di Sarconi” PGI (*Phaseolus vulgaris* L.). *Biology and Life Sciences Forum*. 2020;4(1):57.
7. ISTA. International Rules for Seed Testing (Supplement Rules). *Seed Science and Technology*. 1999;27:25–30.
8. Vitti A, Bevilacqua V, Logozzo G, Marzario S, Gioia T, Nuzzaci M. Response of two local common bean ecotypes of “Fagioli di Sarconi” PGI (*Phaseolus vulgaris* L.) to seed-borne pathogens and environmental change. *Agronomy*. 2021;11(10):1924.

How to cite this article: AB K, SJ M, VS H, DE P, VH T, NH P, et al. Comparative study on detection, isolation and identification of seed-borne mycoflora in different varieties of French bean. *International Journal of Research in Agronomy*. 2026;9(10):611–617. doi: <https://www.doi.org/10.33545/2618060X.2026.v9.i10h.6857>

Authors

Kamble AB

PG Scholar, Department of Plant Pathology, College of Agriculture, Latur VNMKV, Parbhani, Maharashtra, India

Magar SJ

Professor, Department of Plant Pathology, College of Agriculture, Latur VNMKV, Parbhani, Maharashtra, India

Hande VS

PG Scholar, Department of Plant Pathology, College of Agriculture, Latur VNMKV, Parbhani, Maharashtra, India

Pathak DE

PG Scholar, Department of Plant Pathology, College of Agriculture, Latur VNMKV, Parbhani, Maharashtra, India

Tandle VH

PG Scholar, Department of Plant Pathology, College of Agriculture, Latur VNMKV, Parbhani, Maharashtra, India

Patil NH

PhD Scholar, Department of Plant Pathology, VNMKV, Parbhani, Maharashtra, India

Ovhal SS

PG Scholar, Department of Horticulture (Fruit Science), College of Agriculture, Latur-413512, VNMKV, Parbhani, Maharashtra, India